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Effects of Malathion on Microorganisms of an Artificial Salt-Marsh Environment[1]

A. W. Bourquin[2]

ABSTRACT

Laboratory salt-marsh environments were treated with malathion, an organophosphate insecticide, and aerobic heterotrophic bacteria were monitored to determine changes in their microbial ecology. Several physiological activities were assayed in both treated and untreated controls; however, no reliable trends in numbers of these microorganisms were detected. On the other hand, populations of malathion sole-carbon-degrading bacteria increased significantly with increasing treatment levels and in the sediments with repeated treatment. Malathion cometabolizing bacteria increased significantly over the control systems in the water column with increasing treatment levels. Although numbers of malathion-degrading bacteria increased with higher treatment levels or frequency of treatment, these changes had no effect on the total numbers of bacteria from the water or sediment. When an organochlorine insecticide, mirex, was used to treat the ecosystems, essentially no changes in the bacterial populations were detected.

Additional Index Words: microbial-pesticide interaction, degradation, effects of pesticides, microbial activities, mirex.

Estuarine environments are composed of highly complex communities of organisms, some of which are inseparably linked to coastal marshlands. The most productive parts of the estuary are the intertidal and adjacent shallow-water zones (12). Coastal nekton frequently use estuaries as nursery grounds where larval, through adolescent stages, can take advantage of the protection and abundant food. Because early life stages of many important commercial and sport fisheries depend on estuaries, it is economically important to protect these habitats. These saline marshlands are also prime breeding sites for mosquitoes. Recently, massive mosquito control programs have been established to control the adult mosquito in municipal areas and, in many instances, effective treatment has necessitated insecticide application on or near marshes that serve as nursery grounds for a variety of marine species (6). Concern for possible hazards to nontarget species in the salt-marsh has prompted studies to determine the fate and effects of these chemical toxicants.

Like most organophosphates, malathion [S-(1,2-Dicarbethoxyethyl)-O,O-dimethyl dithiophosphate] is relatively unstable in the environment. Degradation of malathion occurs nonenzymatically by hydrolysis (8), and enzymatically by way of phosphatase or carboxyesterase activity or both. The two modes of degradation may be complementary in the breakdown of the insecticide in soil (15). Seventy-two hours following application in an estuary, malathion could not be detected at temperatures above 15.6C (60F); hence, it was reported as completely degraded. However, no mechanism for dissipation was proposed (7). In another study, a high concentration of malathion was observed immediately after application to a salt marsh, but progressively lower concentrations were observed at 24, 33, and 48 hours after application (5). Similarly, when malathion was applied to a marsh, insecticide concentration in marsh water immediately after fogging and after ultralow volume (ULV) applications was 5.2 and 0.49 ppb; only trace amounts (0.1–0.3 ppb) persisted for the first 24 hours (14). This rapid dissipation of parent compound is probably a consequence of both chemical and biological degradation. Malathion, although degraded chemically, can serve as a substrate for microbial metabolism (9). Several species of soil fungi and bacteria capable of attacking malathion have been isolated (11). Similarly, in a study of the relative importance of chemical as opposed to microbiological degradation of malathion in several Mississippi soils, Walker and Stojanovic (16) found microbial degradation to be the chief mechanism of insecticide dissipation in all soils tested.

Despite numerous studies on the fate and effects of malathion in estuarine salt-marsh ecosystems or in soil systems, little information exists concerning its effect on estuarine microbial ecology. The objectives of this study were (i) to determine the effect of malathion on heterotrophic bacteria, and (ii) to compare effects of nonbiode-

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gradable pesticide, mirex (Dodechlorootahydro-1,3-metheno-2H-cyclobuta(cd)diene), on an artificial estuarine microbial ecosystem with those produced by malathion.

MATERIALS AND METHODS

Materials

Malathion, malaoxon, and the carboxylic acid products were obtained from American Cyanamid Co., Princeton, N. J. Mirex was obtained from Allied Chemical Corp., Morristown, N. J. Analytical-grade standards for chromatography were obtained from U. S. Environmental Protection Agency, Pesticides Reference Standards Section, Chemistry Branch, Washington, D. C. All organic solvents used for pesticide extraction were nanograde (Mallinckrodt Chemical Works, St. Louis, Mo.); Acetone and chromatographic solvents were spectroanalyzed grade (Fisher Scientific Co., Fair Lawn, N. J.). Microbial media were purchased from Baltimore Biological Lab., Baltimore, Md.

Experimental Methods

PREPARATION OF LABORATORY ECOSYSTEMS

Sediment and water with attending microorganisms were collected from a salt marsh on Santa Rosa Island, near Gulf Breeze, Fla., and transported to the laboratory in < 0.5 hours. Sediment (sand) from the salt marsh was sieved through a no. 7 mesh screen to remove large particles and facilitate sampling procedures. Sieved sand (7.62 cm deep) was placed into acetone-cleaned battery jars (16 by 16 by 26 cm) and 6.5 liters of marsh water were added. Air was slowly bubbled through a Pasteur pipette, fixed about 2/3 of the water depth, to prevent anaerobiosis or surface slick formation. Water temperature was maintained at 28C (± 1C) and salinity at 20 ‰ (± 2 ‰) for the duration of the experiment. The artificial ecosystems were exposed to a 12-hour light-dark cycle (6,000 lux).

After an initial 72-hour settling period in the jars, water and sediment samples were assayed microbiologically to insure that microbial numbers returned to nominal environmental levels or stabilized at a higher concentration. Once stabilization was ascertained, the pesticide was added and the test period began.

Malathion in acetone (0.5 ml) was added to the water surface of two battery jars at 420 g/ha (field application rate) and at 4.2 kg/ha (10X field rate). The artificial ecosystems were treated every 10 days during two 30-day experiments to simulate a mosquito-control program. Mirex was added to the stabilized ecosystems at 1,000 and 100 µg/liter. The mirex ecosystems were retreated at 20 days after initial treatment.

SAMPLING AND ANALYSES

Ten-milliliter water samples, taken from the middle of the water column, were diluted serially for plate counts. Ten-gram (wet weight) sediment samples were taken with a large-bore sterile pipette and diluted serially for plate counts. Sediment and water samples were taken immediately before treatment, at "0" time (immediately after treatment), and at 1, 3, 7, and 10 days thereafter.

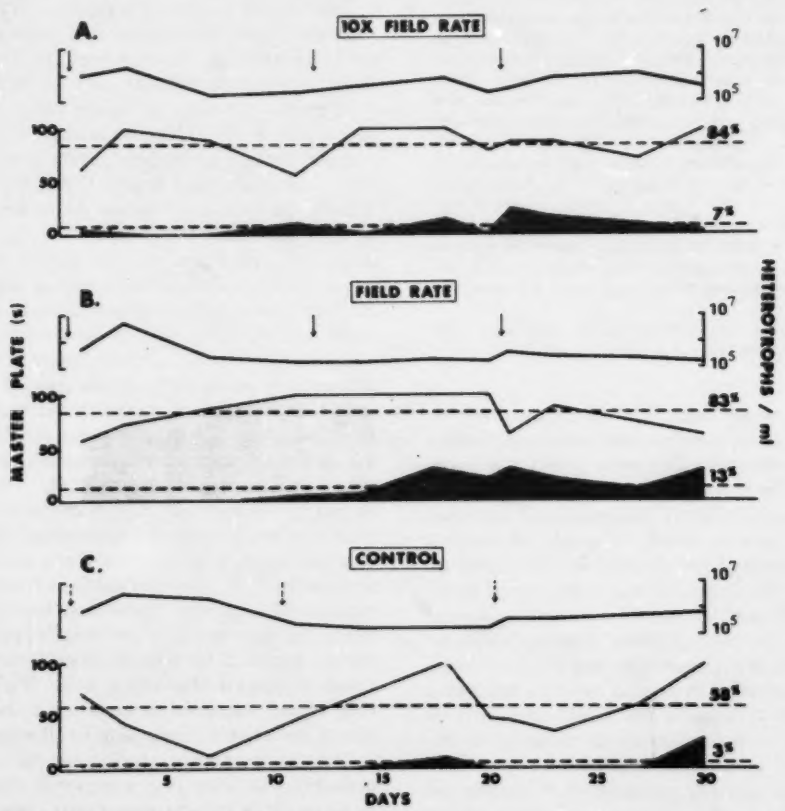

Fig. 1—Average numbers of heterotrophic bacteria from triplicate samples from water column of artificial salt-marsh ecosystem presented as heterotrophs/ml (line), and number of heterotrophic bacteria which are malathion sole-carbon degraders (dark) or malathion cometabolizers (shaded), given as a percentage of the "master-replica-plate." Times of malathion treatment are indicated by arrows; controls are untreated. Dashed line indicates the 30-day average for each category.

Total heterotrophic bacteria were enumerated by plating on Marine 2216 agar (Difco, Detroit, Mich.). Malathion cometabolizing (MCM) bacteria were enumerated in like manner on marine agar plus 50 mg malathion/ml. MCM bacteria were defined as organisms that would not degrade malathion without an additional substrate. Chitinoclastic bacteria were enumerated according to the procedure of Hood and Meyers (10). After 3 days incubation at 28C, select colonies from marine agar plates were checked for hydrolysis of starch, lipid, and casein by the replica-plating technique. Media for these tests were prepared according to Colwell and Wiebe (4). Master plates were chosen from marine agar heterotroph plates that contained 50–100 colonies per plate, representing the best distribution of colony types among all dilutions. Replica plates were incubated at 28C for 4 days, when amylase, lipase, and casein producers were counted.

Microbial sampling from the mirex ecosystems was done similarly. Tengram samples of sediment and water were taken as described for malathion residue analyses at days 0, 7, 10, 13, 20, and 23. Residues were determined as described elsewhere (2, 13). Mirex sample analyses were conducted as described by Borthwick et al. (1). The pH and salinity of the water were determined every 2 days and adjusted to the original in situ parameters.

STATISTICAL ANALYSIS

Data from the artificial ecosystem studies were treated by analysis of variance to determine significant differences (i) between control and experimental cultures over a 30-day treatment period, (ii) during a single treatment period (every 10 days), and (iii) at each treatment level.

RESULTS

After introduction of malathion into the laboratory ecosystems, changes in heterotrophic biomass and physiological indices of the artificial ecosystem were monitored. Figure 1 presents the average number of aerobic heterotrophic bacteria in water columns of duplicate artificial salt-marsh environments: (A) malathion, 10X field application rate (top); (B) malathion, field application rate (middle); and (C) no malathion (bottom, control). Figure 2 presents the same data from sediment sampling. The data represent the percentages of colonies from nutrient marine medium that grew on replicate-test medium. No apparent effects on indigenous microflora resulted from the addition of 0.5 ml acetone to similar experimental ecosystems; no acetone control data were included in this paper. Essentially no differences in total heterotrophic activity were noted between control and experimental cultures of either water (Fig. 1) or sediment (Fig. 2) samples. However, malathion-degrading bacteria in treated water (Fig. 1) were greater than those in control water during the 30-day treatment period. Sole-carbon-degrading bacteria (SCD) increased by 7 and 13%; malathion cometabolizing bacteria (MCM) increased by 25 and 26% over the untreated controls. Statistically, MCM's were

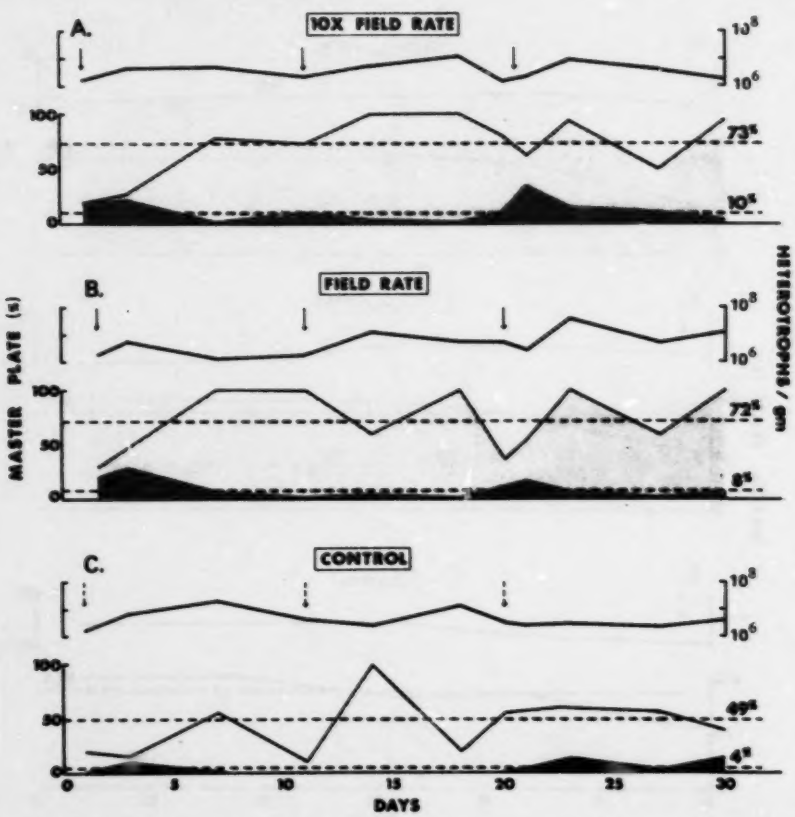

Fig. 2—Average numbers of heterotrophic bacteria from triplicate samples from sediments of artificial salt-marsh ecosystems presented as heterotrophs/gm of wet sediment. Numbers of heterotrophic bacteria that are malathion sole-carbon degraders (dark) or malathion cometabolizers (shaded) given as a percentage of the "master-replica-plate." Dashed line indicates the 30-day average for each category. Times of malathion treatment indicated by arrows; controls are untreated.

significantly different ($\alpha = 0.01$) among treatment levels (10X, 1X, and the control). SCD's in the water increased with the number of treatments. (0, 10, 20 days. $\alpha = 0.05$) but were not significant between treatment levels ($\alpha > 0.1$). At the 10X application rate, only 7% of the heterotrophic bacteria on the master plate were SCD's whereas, at the 1X application rate, 13% were.

Fluctuations in numbers of bacteria were greater in all sediment samples (Fig. 2), but the results were similar to those of the water samples. SCD's increased significantly ($\alpha = 0.10$) among treatment levels (control, 1X, and 10X) and among individual treatment periods (0, 10, and 20 days. $\alpha = 0.05$), i.e., number of treatment.

Numbers of MCM's increased significantly ($\alpha = 0.1$) with frequency of treatment (0, 10, 20 days) at both treatment levels. Although MCM bacteria were not significantly different ($\alpha > 0.1$) at increased levels of treatment (control, 1X, and 10X), MCM's increased in experimental vs. control systems from 49% on the control to 72 and 73% of the heterotrophic bacteria on the master plate from the 1X and 10X treated systems, respectively. Isolates representing malathion sole-carbon-degrading and cosubstrate-degrading bacteria were randomly selected from both type media and assayed for malathion degradation as previously described (2). These results confirm the organisms as malathion utilizers.

Analysis of physiological groups of bacteria affected by the addition by malathion showed no correlation with concentration or frequency of treatment (Table 1). Chitinolytic bacteria were not sufficiently high in number to compare among treatments. Numbers of bacteria that exhibited the functions tested were greater in the sediments than in the water column. No reliable trends were found in the percentages of the test groups in the controls or experiments.

No malathion residues were detected in the water or sediments at days 7, 13, or 23. After the initial treatment of the system, the microorganisms appeared acclimated to malathion breakdown, resulting in a decreased pesticide residence time in the system. Microorganisms selected from MCM's and SCD's were tested for their ability to use malathion. These results are reported elsewhere (2).

To compare the effects on the microbial ecosystem of a recalcitrant insecticide with those of a biodegradable insecticide (malathion), similar artificial environments were challenged with mirex. The data are presented in Fig. 3 (water) and 4 (sediment). No differences among control and treated systems were noted for heterotrophic bacteria in water or sediment samples. However, some decrease in numbers of "mirex-tolerant" bacteria (i.e., bacteria which grew on nutrient medium plus 10 mg mirex/ml) is apparent, especially at the higher treatment level. This decrease

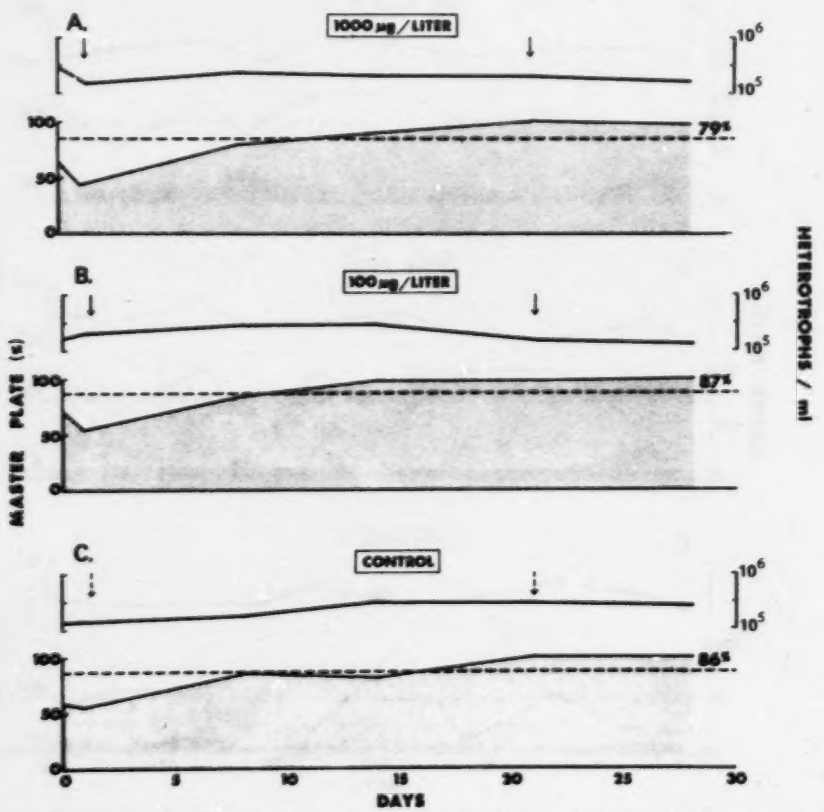

Fig. 3—Average numbers of heterotrophic (solid line) and mirex-tolerant bacteria (shaded) from triplicate samples from water column of artificial salt-marsh ecosystems presented as heterotrophs/ml and percentage of "master-replica-plate." Arrows represent time of mirex treatment; controls are untreated. Dashed line represents the 30-day average for each category.

Table 1—Percentage of heterotrophic populations in malathion-treated laboratory ecosystems

	Sediment samples									Water samples								
	Amylolytic			Proteolytic			Lipolytic			Amylolytic			Proteolytic			Lipolytic		
Day	Control	1X	10X	Control	1X	10X	Control	1X	10X	Control	1X	10X	Control	1X	10X	Control	1X	10X
1	47	11	7	35	14	18	29	1	4	11	1	10	25	2	4	11	8	30
3	52	100	22	44	100	30	14	4	7	35	10	6	1	10	2	35	40	25
7	100	8	17	100	3	25	5	4	2	4	1	8	7	1	82	4	1	1
10	75	18	17	50	18	100	20	3	16	1	5	1	2	18	4	1	1	1
13	100	66	12	100	57	9	7	1	2	1	5	1	1	1	1	1	1	1
17	5	7	14	27	100	17	5	4	5	1	1	1	1	1	1	1	1	1
20	22	8	22	100	7	7	5	2	1	2	1	1	1	4	3	2	1	1
23	30	3	7	36	3	9	1	1	1	1	1	6	2	40	5	1	1	1
27	16	12	15	100	100	17	18	5	9	2	1	1	1	1	4	2	1	1
R†	−1	−0	0	0	−0	−0	0	−0	−1	−0	−1	0	0	−1	0	0	−1	−1

† R = Correlation coefficient.

in bacteria was evident after the initial treatment; however, the populations appeared to recover and were unaffected by the latter treatments. No mirex-degrading bacteria were selected by the replica-plating procedure. The overall effect of mirex on the microbial ecosystem of an artificial salt-marsh environment bears little resemblance to that of malathion, a "softer" organophosphate insecticide. When the artificial ecosystem was treated with mirex, no stimulatory or inhibitory effect, other than the initial treatment, was noted.

DISCUSSION

When applied to a simulated salt-marsh environment, malathion degraded rapidly and evidence for an active involvement by the indigenous bacterial community is shown. In these laboratory environments, numbers of bacteria capable of degrading malathion in the presence of additional nutrients increased in the sediments with repeated application, and increased in the water column, as the level of treatment increased from 0 to 1X, but there

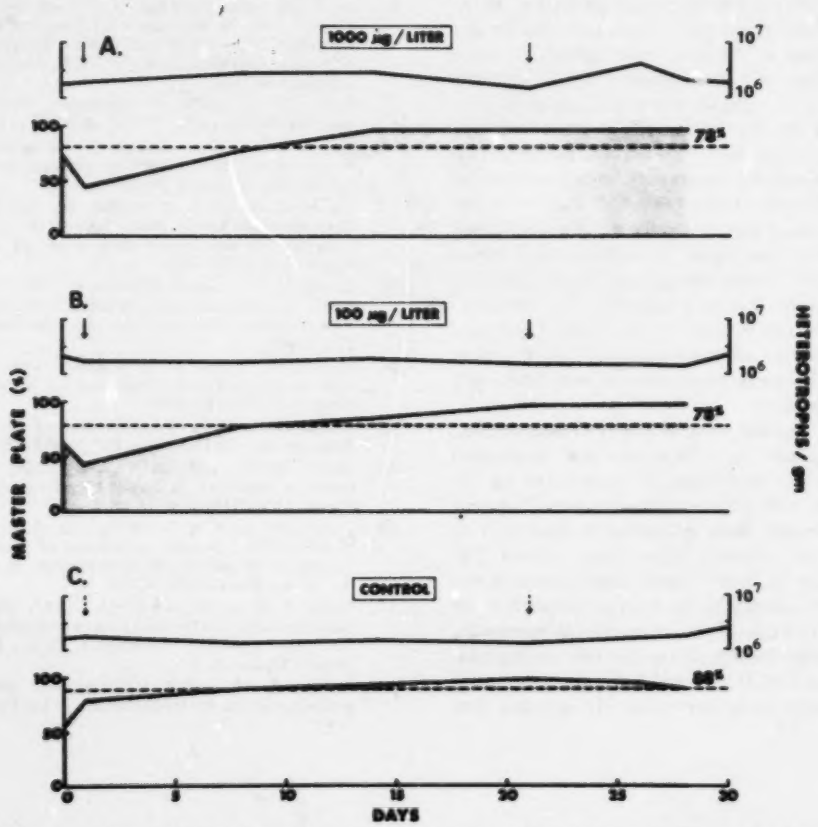

Fig. 4—Average numbers of heterotrophic (solid line) and mirex-tolerant bacteria (shaded) from triplicate samples from sediments of artificial salt-marsh ecosystems and percentage of "master-replica-plate." Arrows indicate times of mirex treatment; controls are untreated. Dashed line represents the 30-day average for each category.

was no additional increase with higher application rates (10X, Fig. 1 and 2). On the other hand, numbers of bacteria that degraded malathion as a sole-carbon-source (SCD's) were linked to the level of treatment in sediments and to the number of treatments in the water column. In either water or sediments, SCD's did not appear to play a significant role in the degradation of malathion, comprising only about 10% of the bacteria that degrade malathion. Other studies have shown that the predominant isolates from salt marshes are malathion cometabolizing rather than malathion sole-carbon-degrading organisms (2). It is possible that the MCM's catalyzed a more rapid degradation of the compound, resulting in a decreased selection of SCD's in the water column. At the same time, the increase of SCD's in the sediment may have resulted from malathion reaching the sediments through precipitation. Malathion, although soluble to 145 mg/liter of distilled water, would be substantially less soluble in water of 15 °/oo salinity. No changes in physiological indices were noted.

If certain groups of bacteria were inhibited by the pesticide or its metabolites, or groups were selected due to competition by other microorganism (i.e., MCM's), these groups were not detected. No reason can be given for the major fluctuations in percentages of the total population exhibiting the functions tested. These fluctuations were so great that no trends could be shown statistically. However, the method of selection has a degree of bias for the predominant organisms at the time of sampling. To keep the number of organisms per plate below 100, the selected master plate may not be representative of all species present in the systems at the time of sampling.

Although mirex is inhibitory to certain bacteria (3), heterotrophic bacteria in the laboratory environments did not reflect any changes attributable to the toxicant. Changes in populations of bacteria were more pronounced when a biodegradable insecticide (malathion) was introduced into the system. These changes can be explained as competitive interactions due to a selection for pesticide-degrading bacteria, rather than to an inhibitory reaction. No changes were noted in the systems treated with mirex; no mirex-degrading bacteria were selected and inhibitory responses were not evident.

Malathion, when applied to salt-marsh environments, should rapidly degrade by chemical and biological mechanisms. Chemical hydrolysis is stimulated by increasing temperature and salinity (9), but would appear to be of less importance than microbial degradation as evidenced by selected cultures from these studies (2). Microbial degradation is more rapid than chemical degradation, apparently mediated by a large proportion of salt-marsh bacteria. In a complex salt-marsh environment, MCM's would probably assume the major role in degradation of malathion, and SCD's would become active only when application rates were excessive. It appears that malathion in salt-marsh environments is rapidly degraded by the indigenous bacterial community, without apparent adverse effect on the microbial community tested here.

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16. ABSTRACT

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